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Effect of fenofibrate in comparison to metformin on the biophysical and biochemical parameters in diabetic albino wistar rats

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***Abstract*---**Introduction: Diabetic endothelial dysfunction is accompanied by increased oxidative stress and upregulated proinflammatory and inflammatory mediators in the endothelial vasculature. Activation of peroxisome proliferator-activated receptor-alpha (PPAR-α) results in antioxidant and anti-inflammatory effects. Aim of this study is to investigate the effect of fenofibrate, a PPAR-α activator, on the oxidative stress, inflammation and its anti-oxidant effect in streptozotocin diabetic rats and to compare the effectiveness of FF with that of Metformin (Met). Material & Methods: This experimental animal study was conducted at animal house. The sample size included 174 albino wistar rats divided into 3 Groups, one

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control groups (C)Diabetic and untreated and two test groups .T1 - Diabetic and treated with metformin 75 mg/kgwt/day) and T2 (T – Diabetics treated with fenofibrate 100 mg/kgwt/day), with 58 rats in each group (29 male & 29 female). All the rats were treated with streptozotocin intra peritoneally and the diabetic state was induced. T1 group was treated with metformin 75 mg/kg/wt/day. The T2 group of rats were treated with Feno fibrate (FF) at a dose of 100 mg/kgwt/day. Blood sample was drawn from retro orbital plexus of animals and the biophysical and biochemical parameters were tested at an interval of 3, 6 and 12-months duration. Comparison was done between the metformin treated control group and fenofibrate treated test group. Results: Test of statistics, one way Analysis of Variance (ANNOVA) was used to compare the groups. Dunnet's test was used to do a multiple compression. Fenofibrate treatment with a dose of 100 mg/kgwt/day was significant in comparison with metformin on the biophysical (body weight), biochemical parameters (RBS (random blood sugar), urea, creatinine, HbA1C,Total cholesterol, Triglycerides, HDL -C, LDL-C, Inflammatory cytokines, TNF Alpha tissue kidney, PPAR Alpha tissue kidney, NfKB tissue kidney and on the oxidative stress (MDA) and on antioxidant status (SOD) in diabetic rats. Conclusion: Our findings suggest that PPARα activation by fenofibrate, generates a protective effect in diabetes induced rats from progression of diabetes and there in preventing the diabetic complications. This may represent a novel treatment strategy along with the existing treatment strategies to limit microvascular injury related to diabetes mellitus.

***Keywords*---**Feno fibrate (FF), Metformin (Met), Streptozotocin (STZ), Biophysical parameters, Biochemical parameters.

Introduction

Diabetes mellitus, is termed as a major epidemic of this century [1], which has increased in incidence by 50% over the past 10 years. The incidence of diabetes is rapidly increasing with estimations suggesting that this number will almost double by 2030 [2]. The greatest increase in prevalence in the near future, however, is expected to occur in Asia, the Middle East [3], and Africa, where it is likely that there will be an 50% increase in diabetes in these parts of the world by 2030 [1]. Type 2 diabetes (T2D) is a chronic metabolic disorder that results from defects in both insulin secretion and insulin action. Elevated rates of basal hepatic glucose production in the presence of hyperinsulinemia are the primary cause of fasting hyperglycaemia; after a meal, impaired suppression of hepatic glucose production by insulin and decreased insulin mediated glucose uptake by muscle contribute almost equally to postprandial hyperglycaemia [4]. Diabetic patients develop vascular complications at a much faster rate in comparison to nondiabetic individuals, and cardiovascular risk is increased up to tenfold [5]. Endothelial dysfunction and oxidative stress play a key role in the pathogenesis of diabetic vascular disease [6]. DM is characterized by hyperglycemia and hyperlipidemia, two cardinal biochemical features associated with inhibition of

endothelial nitric oxide synthase (eNOS), leading to diminished NO production and increased formation of reactive oxygen species (ROS) in endothelial and vascular smooth muscle cells. Besides, impaired expression or activity of some antioxidant enzymes such as superoxide dismutase (SOD) and catalase contributes to the development of endothelial dysfunction in DM by increasing oxidative stress [7]. Endothelial dysfunction accompanied by upregulated proinflammatory and inflammatory mediators is thought to be another contributing factor to the pathogenesis of diabetic vascular complications. Multiple effects of inflammatory cytokines like interleukin-1 (IL-1) and tumor necrosis factorα (TNF-α), which lead to prothrombotic and proinflammatory changes on the vascular endothelium, have been outlined in some reports [8].

Peroxisome proliferator-activated receptors (PPARs) belong to a subfamily of the nuclear receptors involved in glucose and lipid metabolism; the group includes three isotypes encoded by different genes: PPARα, PPARβ/δ, and PPARγ [9]. Peroxisome proliferator-activated receptor-α (PPAR-α) is a hormonal activated nuclear receptor which plays an important role in the course of many vascular diseases such as DM, hypertension, and coronary heart disease [10,11]. In recent publications, it has been clearly demonstrated that activation of PPAR-α leads to an anti-inflammatory effect by reducing plasma concentrations of TNF-α. On the other hand, it produces an antioxidant effect by reducing plasma concentrations of malonyldialdehyde, major indicator of oxidative stress, and by stimulating the expression of SOD, one of the major molecules of antioxidant defense [12,13].

It has been observed that PPARα ligands, including fibrates, reduce myocardial ischemia/reperfusion (I/R) injury in diabetic and nondiabetic animals; this cardio protection might be mediated through anti-inflammatory mechanisms and via the activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (Akt)/NO pathway [14,15,16]. Recently, Barreto-Torres et al. [17] showed that metformin, a widely used antidiabetic drug for T2D, exerts cardio protection in rats with myocardial I/R injury via activation of PPARα. In this context fenofibrate (FF), a third generation fibric acid derivative and a PPAR-α agonist, can be a beneficial choice for the treatment of diabetic vascular complications because of its anti-inflammatory and antioxidant effects. Moreover, FF is a useful drug for the treatment of atherogenic dyslipidemias, producing a substantial decrease in the levels of triglyceride-rich lipoproteins and an increase in high density lipoprotein cholesterol levels. Therefore, the aim of this work was to test the effectiveness of PPARα activators fenofibrate in comparission with metformin exerting an antioxidant, anti oxiditive stress, anti-inflammatory effect and leading to cardioprotection in STZ induced diabetic albino wistar rats.

Material and Methods

Animals

It was an animal based experimental study conducted at Animal House of Faculty of Palamur Bioscience Private Limited for a period of one year.The study was approved by Institutional Animal Ethics Committee (IAEC) Palamur Biosciences Private Limited. CPCSEA Registration Number -1312/PO/ReBiBt-S/ 09/CPCSEA. Animals were obtained from in house bred at Palamur Biosciences Pvt. Ltd.

Experimental design

All the animals were fed by standard rat pellet diet and were allowed for free access to water. The rats were housed in standard cages at a constant temperature (15 ^{0} – 25 ^{0} Centigrade) with fixed 12: 12-hour light-dark cycle. The sample size included 116 albino wistar rats divided into 3 Groups, one control groups (C) and two test groups T1 and T2, with 58 rats in each group (29 male & 29 female). All the rats in the group were subjected to overnight fasting. Next day, all the rats were treated with streptozotocin (55 mg/kgwt) intra peritoneally before use was dissolved in 0.1 M in freshly prepared sodium citrate buffer, pH 4.5, made isotonic by the addition of 0.25M NaCl. [18-20]. 5% of glucose water was given for two days, to prevent drug-induced hypoglycaemic shock. Seven days after the administration of STZ injection, the blood sample was collected from retroorbital plexus and centrifuged at 1000 x g for 20 minutes and the serum (supernatant) was collected. Blood glucose levels were determined by using the commercial kits by semi autoanalyzer the rats with blood glucose levels of 200mg/dl or more were considered as diabetic. Control group was monitored without any treatment. T1 was treated with metformin (75 mg/kgwt/day) by oral gavage. The test group T2 of rats were treated with Feno fibrate (FF) at a dose of 100 mg/kgwt/day by oral gavage.

Sample collection and tissue preparation

Blood sample was collected from retro orbital plexes of eye with the help of hematocrit capillaries (SD-Fine Pvt ltd).Rats were individually caged for 24 hours in metabolic cages and the urine sample was collected. Animals were sacrificed. Left kidney was removed and immediately preserved in 10% buffered formalin solution for histopathological examination.Right kidney was removed and washed with phosphate buffer and then homogenized in a homogenizing buffer (0.1 M phosphate buffer, pH 7.4) using telon homogenizer. The homogenate was centrifuged t 9000g for 20 minutes to remove debris.The supernatant was further centrifuged at 15,000g for 20 minutes and the supernatant was used for various biochemical assays. Following investigations were performed at an interval of 3 ,6 and 12 months duration. Body weight, organ weights (kidney and eye) were measured. Random blood sugar (RBS) mg/dl, Glycosylated hemoglobin (HbA1C %), Urea mg, Creatine mg, Urine albumin, (TC)Total cholesterol, (TG) Triglycerides, High density lipoprotein (HDL-C), Low density lipoprotein (LDL-C), Tumor necrosis factor alpha (Tissue kidney TNFα), ,Peroxisome proliferator activated receptor (PPAR α tissue kidney) nuclear factor kappa light chain enhancer of activated B cells (NfKB tissue kidney), Malonaldehyde (MDA), Super oxide dismutase (SOD).

Determination of RBS and HbA1C

RBS in mg/dl was estimated by semiauto analyser using erba blood glucose kit. Method used was Trinder's method [21]. Standard procedure as per the instruction manual was followed. HbA1C % was measured by HPLC method [22,23] in Bio-Rad D 10 instrument. Standard procedure as per the instruction manual was followed.

Evaluation of kidney function

Urea in mg was estimated by semiauto analyser using erba blood urea kit. Method used was Urease-GLDH-fixed time method [24,25]. Creatine in mg was estimated by semi autoanalyzer using erba kit. Method followed was Jaffe's method [26]. Urine protein in mg/dl was estimated by nephelometry method. Standard procedure as per the instruction manual was followed [27]

Evaluation of lipid profile

TC was estimated by CHOD-PAP method in semi autoanalyzer. This reagent is based on the formulation of Allain et al and the modification of Roeschlau with further improvements to render the reagent stable in solution [28,29]. TG was estimated by GPO-Tinder, end point method in semiauto analyser. This reagent is based on the method of Wako and the modifications by McGowan et al and Fossati et al [30,31]. HDL-C was estimated by phosphotungstic acid method in semi auto analyser [32-34]. LDL-C was calculated by using Friedewald formula [35-39]. Standard procedure as per the instruction manual was followed

Evaluation of inflammatory cytokines

Tumor necrosis factor alpha (Serum TNFα) was estimated in ELISA reader.It is a kit based on sandwitch enzyme- linked immune -sorbent assay technology[40]. NfKB serum was estimated in ELISA reader. It is a kit based on sandwitch enzyme- linked immune -sorbent assay technology [41]. PPAR α in tissue kidney homogenate was estimated in ELISA reader. It is a kit based on sandwitch enzyme- linked immune -sorbent assay technology [42,43]. TNF α in tissue kidney homogenate was estimated by ELISA reader. [44]. NfKB in tissue kidney homogenate was estimated by ELISA reader. Standard procedure as per the instruction manual was followed [45].

Evaluation of lipid peroxidation

MDA levels were measured in the serum by spectrophotometric method. Standard procedure as per the instruction manual was followed [46-48].

Evaluation of anti-oxidant status

SOD levels were estimated by spectrophotometer. Standard procedure as per the instruction manual was followed [49,50].

Statistical Analysis

Data has been entered in MS XL software. Frequency distribution tables have been calculated and tabulated. Test of statistics, one way Analysis of Variance (ANNOVA) was used to compare the groups. Dunnet's test was used to do a multiple compressions and test of significance was tested at $p<0.001$

Table 1
Comparison of the Biophysical and Biochemical parameters among diabetic untreated controls (C) and test T1 Diabetic treated controls with metformin 75 mg/kg/wt/day and T2 Diabetic group treated with fenofibrate with 100 mg/kgwt/day for a period of 3 months

S.no	PARAMETERS	C Untreated 3m Mean ± SE	T1 Treated 3m	T2 FF100 Test 3 m
1.	Body wt(gms)	188.6±1.601	195.5±6.054	105.8±6.054
2.	RBS g/dl	213.1±9.471	187.1±3.206	186.0±3.206
3.	HbA1C %	6.249±0.197	7.154±0.156	6.993±0.162
4.	Urea mg	35.49±1.86	36.96±1.198	36.35±1.198
5.	Creatine mg	0.904±0.080	0.801±0.025	0.810±0.025
6.	(TC)Total cholesterol	89.50±2.33	79.14±1.606	78.35±1.606
7.	(TG) Triglycerides	133.5±1.990	118.2±2.991	123.4±2.991
8.	HDL-C	19.06±0.389	16.12±0.446	16.37±0.446
9.	LDL-C	43.75±2.33	39.39±1.840	37.30±1.840
10.	PPAR Alpha tissue kidney	2.270±45.13	2.413±0.164	2.309±0.164
11.	TNF Alpha tissue kidney	76.01±2.330	71.86±1.522	73.14±1.522
12.	NfKB tissue kidney	6.768±0.176	5.692±0.224	5.915±0.224
13.	MDA	10.63±45.71	7.106±0.182	6.925±0.182
14.	SOD	2.526±0.124	2.631±0.193	2.702±0.193

Table 2
Comparison of the Biophysical and Biochemical parameters among diabetic untreated controls (C) and test T1 Diabetic treated controls with metformin 75 mg/kg/wt/day and T2 Diabetic group treated with fenofibrate with 100 mg/kgwt/day for a period of 6 months

S.no	PARAMETERS	C Untreated 6 m Mean ± SE	T1 Treated 6 m	T2 FF100 Test 6 m
1.	Body wt(gms)	185.6±1.0	232.6±8.58	138.5±6.169
2.	RBS g/dl	298.07±7.54	163.48±1.28	164.5±3.323
3.	HbA1C %	7.12±0.15	6.38 ±0.10	6.330±0.1621
4.	Urea mg	49.55±1.50	31.14±1.05	30.39±1.242
5.	Creatine mg	1.38±0.08	0.738±0.012	0.7472±0.026
6.	(TC)Total cholesterol	125.56±1.41	66.59±1.15	69.25±1.664
7.	(TG) Triglycerides	146.55±1.16	108.40±1.59	110.3±3.100
8.	HDL-C	16.68±0.35	19.05±0.35	18.80±0.4630
9.	LDL-C	79.56±1.46	25.86±1.267	28.40±1.907
10.	PPAR Alpha tissue kidney	1.80±0.03	2.70±0.133	2.625±0.170
11.	TNF Alpha tissue	107.7±2.78	69.03±1.098	69.47±1.577

	kidney			
12.	NfKB tissue kidney	7.58±0.13	3.32±0.181	3.581±0.232
13.	MDA	12.40±0.23	6.06±0.10	6.087±0.1889
14.	SOD	1.80±0.05	3.49±0.109	3.240±0.200

Table 3
Comparison of the Biophysical and Biochemical parameters among diabetic untreated controls (C) and test T1 Diabetic treated controls with metformin 75 mg/kg/wt/day and T2 Diabetic group treated with fenofibrate with 100 mg/kgwt/day for a period of 12 months

S.no	PARAMETERS	C Untreated 12 m Mean ± SE	T1 Treated 12 m	T2 FF100 Test 12 m
1.	Body wt(gms)	172.32±1.13	267.64±4.049253	267.64±4.049253
2.	RBS g/dl	386.03±7.56	136.62±1.356256	136.62±1.356256
3.	HbA1C %	8.64±0.095	5.36±0.070969	5.36±0.070969
4.	Urea mg	67.47±1.15	21.40±0.472228	21.40±0.472228
5.	Creatine mg	1.80±0.065	0.60±0.016502	0.60±0.016502
6.	(TC)Total cholesterol	143.29±1.48	58.41±0.77563	58.41±0.77563
7.	(TG) Triglycerides	155.23±1.19	95.197±1.358433	95.197±1.358433
8.	HDL-C	15.15±0.135	21.105±0.291852	21.105±0.291852
9.	LDL-C	97.09±1.376	18.27±0.84227	18.27±0.84227
10.	PPAR Alpha tissue kidney	74.11±46.112	3.96±0.066577642	3.96±0.066577642
11.	TNF Alpha tissue kidney	122.17±1.502	59.20±0.807594282	59.20±0.807594282
12.	NfKB tissue kidney	8.46±0.096	2.38±0.103634064	2.38±0.103634064
13.	MDA	14.48±0.275	3.69±0.096724	3.69±0.096724
14.	SOD	1.17±0.027	4.47±0.10	4.47±0.10778667

Results: Table1, 2 and 3

Body weight

When compared with the (C) untreated diabetic rats (188.6±1.601), there was increase in the body with in the group treated with metformin (T1)(195.5±6.054) and significant decrease in weight in the group treated with fenofibrate(T2) (105.8±6.054) ($p<0.0001$) for a period of 3 months. In 6 months period, when compared with the C group (185.6±1.0),there was significant increase in the body weight in T1 group (232.6±8.58) and significant decrease in T 2 group (138.5±6.169).During a period of 12 months, when compared with group C (172.32±1.13), the body weight in group T1 (267.64±4.049253) and T2 increased significantly (267.64±4.049253)

RBS

When compared with the (C) untreated diabetic rats (213.1±9.471), there was significant decrease in the RBS with in the group treated with metformin T1(187.1±3.206) and significant decrease in RBS in the group treated with fenofibrate(T2) (187.1±3.206) ($p<0.0001$) for a period of 3 months. In 6 months, period, when compared with the C group (298.07±7.54), there was significant decrease in the RBS in T1 group (163.48±1.28) and significant decrease in T 2 group (164.5±3.323). During a period of 12 months, when compared with group C (386.03±7.56), the RBS in group T1 (136.62±1.356256) and T2 decreased significantly (136.62±1.356256)

HbA1C

When compared with the (C) untreated diabetic rats (6.249±0.197), there was slight increase in the HbA1C with in the group treated with metformin T1(7.154±0.156) and slight increase in HbA1C in the group treated with fenofibrateT2 (7.154±0.156) for a period of 3 months. In 6 months, period, when compared with the C group (7.12±0.15), there was slight decrease in the HbA1C in T1 group (6.38 ±0.10) and slight decrease in T 2 group (6.330±0.1621). During a period of 12 months, when compared with group C (8.64±0.095), the HbA1C in group T1 (5.36±0.070969) and T2 (5.36±0.070969) is decreased.

Kidney function parameters
Urea

When compared with the (C) untreated diabetic rats (35.49±1.86), there was slight increase in the urea with in the group treated with metformin T1(36.96±1.198) and slight increase in urea in the group treated with fenofibrateT2 (36.35±1.198) for a period of 3 months. In 6 months, period, when compared with the C group (49.55±1.50), there was slight decrease in the urea in T1 group (31.14±1.05) and slight decrease in T 2 group (30.39±1.242). During a period of 12 months, when compared with group C (67.47±1.15), the urea in group T1 (21.40±0.472228) and T2 (21.40±0.472228) is decreased.

Creatinine

When compared with the (C) untreated diabetic rats (0.904±0.080), there was slight decrease in the creatinine within the group treated with metformin T1(0.801±0.025) and slight decrease in creatinine in the group treated with fenofibrateT2 (0.810±0.025) for a period of 3 months. In 6 months, period, when compared with the C group (1.38±0.08), there was slight decrease in the creatinine in T1 group (0.738±0.012) and slight decrease in T 2 group (0.7472±0.026). During a period of 12 months, when compared with group C (1.80±0.065), the creatinine in group T1 (0.60±0.016502) and T2 (0.60±0.016502) is decreased.

Lipid profile
Total Cholesterol

When compared with the (C) untreated diabetic rats (89.50±2.33), there was slight decrease in the TC within the group treated with metformin T1(79.14±1.606) and slight decrease in TC in the group treated with fenofibrateT2 (78.35±1.606) for a period of 3 months. In 6 months, period, when compared with the C group (125.56±1.41), there was slight decrease in the TC in T1 group (66.59±1.15) and slight decrease in T 2 group (69.25±1.664). During a period of 12 months, when compared with group C (143.29±1.48), the TC in group T1 (58.41±0.77563) and T2 (58.41±0.77563) is decreased.

HDL-C

When compared with the (C) untreated diabetic rats (19.06±0.389), there was slight decrease in the HDL-C within the group treated with metformin T1(16.12±0.446) and slight decrease in HDL-C in the group treated with fenofibrateT2 (16.37±0.446) for a period of 3 months. In 6 months, period, when compared with the C group (16.68±0.35), there was slight increase in the HDL-C in T1 group (19.05±0.35) and slight increase in T 2 group (18.80±0.4630). During a period of 12 months, when compared with group C (15.15±0.135), the TC in group T1 (21.105±0.291852) and T2 (21.105±0.291852) is significantly increased.

LDL-C

When compared with the (C) untreated diabetic rats (43.75±2.33), there was slight decrease in the LDL-C within the group treated with metformin T1(39.39±1.840) and slight decrease in LDL-C in the group treated with fenofibrateT2 (37.30±1.840) for a period of 3 months. In 6 months, period, when compared with the C group (79.56±1.46), there was significant decrease in the LDL-C in T1 group (25.86±1.267) and significant decrease in T 2 group (28.40±1.907). During a period of 12 months, when compared with group C (97.09±1.376), the LDL-C in group T1 (18.27±0.84227) and T2 (18.27±0.84227) is significantly decreased.

Inflammatory cytokine
TNF α tissue homogenate(kidney)

When compared with the (C) untreated diabetic rats (76.01±2.330), there was slight decrease in the TNF α within the group treated with metformin T1(71.86±1.522) and slight decrease in TNF α in the group treated with fenofibrateT2 (73.14±1.522) for a period of 3 months. In 6 months, period, when compared with the C group (107.7±2.78), there was slight decrease in the TNF α in T1 group (69.03±1.098) and slight decrease in T 2 group (69.47±1.577). During a period of 12 months, when compared with group C (122.17±1.502), the TNF α in group T1 (59.20±0.80 and T2 (59.20±0.80) is significantly decreased.

NfKB tissue homogenate (kidney)

When compared with the (C) untreated diabetic rats (6.768±0.176), there was slight decrease in the NfKB within the group treated with metformin

T1(5.692±0.224) and slight decrease in NfKB in the group treated with fenofibrateT2 (5.915±0.224) for a period of 3 months. In 6 months, period, when compared with the C group (7.58±0.13), there was significant decrease in the NfKB in T1 group (3.32±0.181) and significant decrease in T 2 group (3.581±0.23). During a period of 12 months, when compared with group C (8.46±0.096), the NfKB in group T1 (2.38±0.10) and T2 (2.38±0.10) is significantly decreased.

PPAR Alpha tissue homogenate (kidney)

When compared with the (C) untreated diabetic rats (2.270±45.13), there was slight increase in the NfKB within the group treated with metformin T1(2.413±0.164) and slight increase in NfKB in the group treated with fenofibrateT2 (2.309±0.164) for a period of 3 months. In 6 months, period, when compared with the C group (1.80±0.03), there was significant increase in the NfKB in T1 group (2.70±0.133) and significant increase in T 2 group (2.625±0.170). During a period of 12 months, when compared with group C (8.46±0.096), the NfKB in group T1 (2.38±0.103) and T2 (2.38±0.103) is significantly increased.

Oxidative stress
MDA

When compared with the (C) untreated diabetic rats (10.63±45.71), there was significantly decreased in the MDA within the group treated with metformin T1(7.106±0.182) and significantly decreased in MDA in the group treated with fenofibrateT2 (6.925±0.182) for a period of 3 months. In 6 months, period, when compared with the C group (12.40±0.23), there was significant decrease in the MDA in T1 group (6.06±0.10) and significant decrease in T 2 group (6.087±0.1889). During a period of 12 months, when compared with group C (14.48±0.275), the MDA in group T1(3.69±0.096724) and T2 (3.69±0.096724) is significantly decreased.

SOD

When compared with the (C) untreated diabetic rats (2.526±0.124), there was slight increase in the SOD within the group treated with metformin T1(2.631±0.193) and slight increase in SOD in the group treated with fenofibrateT2 (2.702±0.193) for a period of 3 months. In 6 months, period, when compared with the C group (1.80±0.05), there was significant increase in the SOD in T1 group (3.49±0.109) and significant increase in T 2 group (3.24±0.200). During a period of 12 months, when compared with group C (1.17±0.027), the SOD in group T1 (4.47±0.10) and T2 (4.47±0.10) is significantly increased.

Discussion

Our study demonstrated that fenofibrate in comparison with metformin is effective in controlling the glycaemic levels, preserving the kidney function, normalizing the lipid profile, decreasing the inflammatory cytokines, decreasing the oxidative stress and increasing the anti-oxidant status.In our study, the body weight of untreated diabetic group was reduced. over a period of one year , when

compared with metformin an fenofibrate treated rats.This data indicated that treatment of diabetic rats by metformin and fenofibrate had no inhibitory effect on body weight reduction in diabetic rats. Our results were in accordance with the results of similar previous studies.[51-53] Our results suggested that there was a significance decrease in the blood glucose and HbA1C levels in the groups treated with metformin and fenofibrate, when compared with untreated diabetic group. metformin exerts its glucose-lowering (hypoglycemic) effect by suppressing hepatic glucose production. [54,55]. FF is one of the major drugs used in the treatment of dyslipidemia, and it has recently been reported that FF decreases serum levels of cholesterol and triglyceride in STZ-induced diabetic rats [56] and it produces a considerable decrease in serum triglyceride levels. a moderate reduction in LDL cholesterol levels, and a significant enhancement in HDL cholesterol concentrations in a model of diabetic dyslipidemia [57].

This is similar to the findings in our study. All these effects of FF have been attributed to the activation of PPAR-α by FF.Our study showed increased levels of PPAR-α in group treated with fenofibrate ,treated with metformin than with untreated diabetic group.Our study showed there was a significant decrease in TNF α levels in the groups treated with fenofibrate and metformin. This was similr to the findings of Tian-Lun Yang who has reported that FF reduces serum TNF-α levels of rats with LDL-induced endothelial dysfunction [58]NF-κβ plays key role in pathogenesis of vascular complications of diabetes. Persistent hyperglycemia activates NF-κβ that triggers expression of various cytokines, chemokines and cell adhesion molecules. Over-expression of TNF-α, interleukins, and other pro-inflammatory proteins and pro-apoptotic genes by NF-κβ is key risk factor in vascular dysfunction. Inhibition of NF-κβ pro-inflammatory pathway is upcoming novel target for management of vascular complications of diabetes. Our findings showed a decrease in the levels of NFkB in the groups treated with metformin and fenofibrate, thus extending a protective action against diabetic complications.Our findings were similar with the results of Yeh PT, Huang and YH, Chang SW et al[58].

SOD is an important defense enzyme which neutralizes the effect of superoxide anion during the oxidative stress in the tissues. Oxidative stress generally causes damage to the membrane polyunsaturated fatty acids (PUFA) leading to generation of malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS). Several studies have indicated an increase in serum TBARS and a decrease in plasma SOD activity signifying an imbalance between the prooxidant and antioxidant states in the body, leading to an imbalance in systemic redox status [59], There was decrease in the MDA levels in groups treated with metformin and FF, signifying the decrease in oxidative stress.There was increase in the antioxidant status(SOD) in the group treated with metformin and fenofibrate.

Limitations

Due to the ethical issues, the diabetes free rats were not sacrificed and did not consider as controls. Studies with combination of fenofibrate, metformin and natural alkaloids are anticipated as these will reduce the cost burden on the

society in the treatment of diabetic patients and its related micro and macrovascular complications.

Conclusion

In our study we demonstrated that fenofibrate in comparison with metformin, at low doses, generates protective action and prevent the diabetic micro vascular complications in relation to diabetes in rats with STZ induced diabetes. This is probably through PPARα activation, as fenofibrate is PPARα agonist. These findings may represent a novel treatment strategy to limit complications in patients with type 2 diabetes mellitus.

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Conflict of interest – No conflict of interest declared

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